The Emerging BFRs Hexabromobenzene (HBB), Bis(246-tribromophenoxy)ethane (BTBPE), and Decabromodiphenylethane (DBDPE) in UK and Irish Foods

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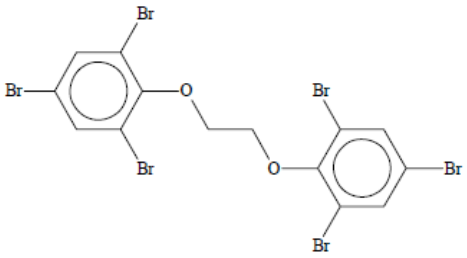
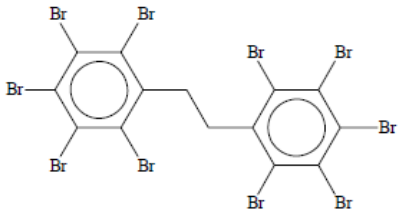
Introduction

There is very little information on the occurrence of BFRs (other than PBDEs, HBCD and TBBA) and PBDD/Fs in food. This is perhaps unsurprising given the relatively recent recognition of the global environmental distribution of these pollutants (D'Silva et al, 2004), and the fact that analytical capability for the reliable measurement of these contaminants is limited. Unlike chlorinated pollutants, BFRs and brominated dioxins are more susceptible to degradation, interconversion and adsorption during analysis and these are important considerations in any analytical methodology used for reliable measurement. Methodology for the analysis of three BFR compounds recently identified by the European Food Safety Authority (EFSA, 2006) as emerging contaminants - Hexabromobenzene (HBB), Bis(246-tribromophenoxy)ethane (BTBPE), and Decabromodiphenylethane (DBDPE) was developed and validated. The methodology was applied to a selection of UK and Irish food samples.



Bis(246-tribromophenoxy)ethane (BTBPE)



Decabromodiphenylethane (DBDPE)

Methodology

The methodology was based on internal standardisation using ^{13}Carbon labelled surrogates of the three compounds and measurement by high resolution GC coupled to high resolution mass spectrometry (GC-HRMS).

Extraction and clean-up

Aliquots of freeze-dried and homogenized sample (5 to 10 g) were extracted after fortification and equilibration with a known amount of $^{13}C_{12}$ labeled surrogates (in typically 50 µL). Extracts were pre-treated with 50g of H_2SO_4 modified silica (1:1, w:w) in 100 ml of n-hexane. The mixture was frequently agitated to prevent setting and continuously swirled to ensure good contact between the matrix and the suspended acid. The mixture was quantitatively transferred to the top of a multi-layer column (70 x 600 mm) packed from top to bottom with; 30 g of anhydrous sodium sulphate, 25 g of H_2SO_4 modified silica gel as described above, 50 g of base modified silica gel (YMC Gel, Kyoto, Japan; prepared by mixing in the ratio 3:1, 5 M KOH in methanol:silica and allowing evaporation of methanol and stabilization for 24 hours), 10 g of sodium sulphate and silanised glass wool. The column was eluted with hexane (100 mL) and dichloromethane:hexane (40:60 v/v, 200 mL). The eluate was quantitatively split into two equal parts, one of which was concentrated and solvent

exchanged to ~1 ml hexane and treated with concentrated H_2SO_4, followed by a wash with de-ionised water. The treated extract was then chromatographed on a 5.6 g activated Florisil™ column (activated by baking at 150^{0}C for >16h) which was eluted with 10 ml of hexane to waste, followed by 60ml of dichloromethane:hexane (40:60 v/v). This fraction was concentrated and solvent exchanged to the volume of the internal sensitivity standard (25 µl of nonane containing $^{13}C_{12}$ -PBDE 139 at a nominal concentration of 100 ng/ml)

GC-HRMS

The extracts were analysed by GC-high resolution mass spectrometry (GC-HRMS) performed on a Micromass Autospec Ultima instrument fitted with a Hewlett Packard 6890N gas chromatograph and a CTC A200SE autosampler. The gas chromatograph was fitted with a 15 m ZB5-MS column (Zebron, Phenomenex), operated using the following oven temperature programme: 3 min at 60^{0}C, 20^{0}C/min to 205^{0}C for 21 min, then 66^{0}C/min to 325^{0}C for 10 min. Injections (10 µl) were made with a PTV injector in constant flow mode using the following transfer programme: 3 min at 60^{0}C, 12^{0}C/sec to 320^{0}C for 3 min, then 12^{0}C/sec to 350^{0}C. The GC-MS interface was set to 280°C. The mass spectrometer used electron ionisation and operated at a resolution of ~ 7000 - 8000 (based on peak width at 10% of peak height) with focussing optimised prior to each run. Selected ion monitoring was employed, using the two most intense ions below m/z 850 for each analyte.

Rationalization of the mass spectral output and processing to calculate the quantity of each compound present was performed using Masslynx 3.5 software supplied by Micromass. These data were transcribed to Microsoft Excel for collation and quantification of concentration data.

Quality Control and Validation

In general terms, the extraction and purification methodology described above is similar to, and is based on the methodology used at FERA (Fernandes et al, 2004; Fernandes et al, 2007) for dioxin and brominated contaminant analysis in that it uses cold solvent extraction and acid hydrolysis of the food matrices, followed by purification using adsorption chromatography and measurement by HRMS. It is different in that the method does not use of activated carbon. The use of these techniques has been peer-reviewed and the methodology has been used successfully over many years for the measurement of chlorinated dioxins, furans and PCBs, and more recently for brominated dioxins, furans and biphenyls.

The use of high resolution mass spectrometry (HRMS) confers a high degree of measurement specificity as well as sensitivity. Method limits of detection are typically of the order of ~0.01 µg/kg for HBB and BTBPE and 0.06 µg/kg for DBDPE on a whole weight basis. The use of ^{13}Carbon labelled surrogates for each of the 3 compounds is a practice that is well-established for environmental contaminant analysis and gives a high level of method control. This results in good reproducibility and replicate measurements on the same matrix have shown an average precision of around 10 % as defined by the relative standard deviation. The accuracy of the measurement has been investigated by the successful analysis of food matrices fortified at different levels, returning concentrations that were in agreement with the fortified values. There are no available reference materials (RMs) for these compounds, but an in-house reference material (fortified sunflower oil) investigated during the course of this work yielded data that was consistent with expected levels.

Analytical recoveries for HBB, BTBPE and DBDPE were based on the incurred ^{13}Carbon labelled surrogates, and were typically within the range 40-80%, which reflects the lability of highly brominated organic molecules. Concentrations reported here are corrected for recovery.

The measurement by GC-HRMS was linear (regression coefficient > 0.995) for all the three analytes over the concentration ranges reported.

For HBB, BTBPE and DBDPE, the expanded uncertainty (coverage factor of 2) (Ellison et al, 2000) for detected compounds was typically around 50% and rising to ~250% for values near the LOD. Reporting limits for HBB and BTBPE were 0.01 μg/kg on a whole weight basis rising to 0.06 μg/kg for DBDPE.

Results and Discussion

HBB and DBDPE were not detected in any foods, whilst BTBPE occurred in some samples. Reporting limits for HBB and BTBPE were 0.01 μg/kg on a whole weight basis rising to 0.06 μg/kg for DBDPE. Little is known about the local production and use of BTBPE – it was intended as a replacement for the banned Octa-BDE. Occurrence in sediment and biota have been documented for North America (Hoh et al 2005; Gauthier et al, 2005), and more proximately, levels of around 0.11 ng/g have been detected in birds eggs from the Faroe Islands (Karlsson et al, 2005). HBB and DBDPE were not found in any samples. This is consistent with reports on the relatively low usage of DBDPE in the UK and occurrence in sediments/sludges (Ricklund et al, 2008). In general, the concentrations of most of the analytes reported here, reflect the utilisation of the various BFRs and the occurrence of brominated contaminants in the environment. There are no maximum permitted limits specified for any of these contaminants and this is perhaps a reflection on the lack of comprehensive data on toxicology and human exposure for these emerging contaminants.

Samples analysed and results for BTBPE where found (whole weight; fat weight, μg/kg):

- *UK sourced food samples (115 samples):*

Meat: Best braising steak; **Rump steak for braising (0.02; 0.56);** Boneless leg of pork; **British pork boneless leg roast (0.06; 0.55);** Boneless shoulder of lamb; **Rolled shoulder of lamb (0.01; 0.05)**; Turkey breast; **Boneless British turkey breast joint (0.03; 1.76);** Fresh chicken legs – boneless; Boneless chicken thighs; Venison haugh joints; Venison fillet.

Meat products and offal: **New Zealand sliced lambs liver (0.03; 0.42);** Lambs liver; Venison liver; Lambs kidney; Pigs kidney; **Lambs kidney (0.02; 0.54); Pork liver (0.03i; 0.81i)**; Ox kidney; Ox liver; British pork sliced liver; **Chicken liver (0.04; 0.75)**; Chicken livers; Ox liver; Traditional lamb sliced liver; Wild venison liver; Farmed red deer liver; Lambs liver; Duck liver pâté with wine; English lamb hearts; Black pudding; Lincolnshire sausages; Newmarket sausages; Premium pork sausages; Cumberland pork sausages; **Scotch beef quarterpounders economy burgers (0.02; 0.10).**

Fish and fish products: Organic boned Scottish salmon fillets; Wild Alaskan salmon fillets (2 samples); Wild Atlantic salmon (2 samples); **Farmed salmon fillet (0.03; 0.26);** Lochmuir Scottish salmon portions; Prime boneless salmon fillets; Rainbow trout; Welsh whole rainbow trout; Mirror carp; **Whole mackerel (0.03; 0.30);** Whole mackerel; Whole mackerel (gutted by fishmonger); Cornish mackerel; Whole herring; **Whole herring (0.06;0.25);** Herring (filleted by fishmonger, 2 samples); Whole Cornish sardines; Whole Cornish sardines – frozen; Cod fillet; **Haddock fillets (0.01; 0.83); Whole lemon sole (0.04; 3.33)**; Dover sole; Plaice fillets (2 samples); Cod fillet; **Whitebait (0.07; 0.77);** Whitebait; Sprats; English sprats; Cooked prawns; Dressed Whitby crab; Smoked eel (2 samples); Eels; Jellied eels.

Milk, cheese and eggs: Pasteurised ewes milk; Organic milk; Vintage extra mature cheddar cheese; Welsh medium Cheddar cheese; Somerset goat's cheese; Duddleswell sheep milk cheese; Cornish brie; Somerset brie; Medium half fat cheese food slices; Cheese spread; Large eggs; Organic free range

eggs; Free range eggs – large; **Free range organic eggs (0.03; 0.29);** Free range eggs; free range duck eggs; Free range duck eggs.
Other: Mushy peas; Cauliflower; Rooster potatoes; Jersey potatoes in water; **British white potatoes (0.01; 2.50);** Carrots; Sweetcorn; Red onions; Spinach; Swede; British parsnips; British tomatoes; Leeks; Blackcurrant coulis; **Organic extra jam handmade strawberry preserve (0.18; 60.00)**; Crispy oven fries; Mini Pringles savoury snack; **Cheese & onion flavour potato crisps (0.04; 0.12);** Pure sunflower oil; Superfast Oats; Wholemeal bread; Olive oil; Pure corn oil.

i – interference prevented quantification and value given represents maximum possible concentration

- *Irish sourced food samples (100 samples):*

None of the Irish food samples analysed were found to contain HBB, BTBPE or DBDPE. These were:

Milk (30 samples sourced from around the country); Liquid egg (20 samples from around the country); Bovine (beef) fat – 8 composite samples each prepared from 10 individual samples; Ovine (lamb) fat – 10 composite samples each prepared from 10 individual samples; Porcine (pork) fat – 6 composite samples each prepared from 10 or 20 individual samples; Avian (chicken(12) or duck(2)) fat – 14 composite samples each prepared from 10 -40 individual samples; bovine liver – 2 composite samples each prepared from 10 individual samples; ovine liver – 1 composite samples prepared from 10 individual samples plus 2 samples obtained from retail stores; porcine liver – 2 composite samples each prepared from 10 individual samples; avian liver – 3 composite samples each prepared from 10 - 40 individual samples; equine (horse) liver– 2 composite samples each prepared from 10 individual samples.

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